



Metabolic injury in a variable rat model of post–status epilepticus

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SUMMARY

Objective: In vivo studies of epilepsy typically use prolonged status epilepticus to generate recurrent seizures. However, reports on variable status duration have found discrete differences in injury after 40–50 min of seizures, suggesting a pathophysiologic sensitivity to seizure duration. In this report we take a multivariate cluster analysis to study a short duration status epilepticus model using in vivo 7T magnetic resonance spectroscopy (MRS) and histologic evaluation.

Methods: The Hellier Dudek model was applied with 45 min of status epilepticus after which the animals were imaged twice, at 3 days and 3 weeks post–status epilepticus. Single voxel point resolved spectroscopy (PRESS) MRS was used to acquire data from the dentate gyrus and CA3 region of the hippocampus, assessing metabolite ratios to total creatine (tCr). In a subset of animals after the second imaging study, brains were analyzed histologically by Nissl staining.

Results: A hierarchical cluster analysis performed on the 3-day data from 21 kainate-treated animals (dentate gyrus voxel) segregated into two clusters, denoted by KM (more injured, $n = 6$) and KL (less injured, $n = 15$). Although there was no difference in kainate dosing or seizure count between them, the metabolic pattern of injury was different. The KM group displayed the largest significant changes in neuronal and glial parameters; the KL group displayed milder but significant changes. At 3 weeks, the KL group returned to normal compared to controls, whereas the KM group persisted with depressed *N*-acetyl aspartate (NAA)/tCr, glutamate/tCr, and increased inositol/tCr and glutamine/tCr. The classification was also consistent with subsequent histologic patterns at 3 weeks.

Significance: Although a short status period might be expected to generate a continuous distribution of metabolic injury, these data show that the short Hellier Dudek model appears to generate two levels of injury. The changes seen in segregated groups persisted into 3 weeks, and can be interpreted according to neuronal and glial biomarkers consistent with histology results.

KEY WORDS: Glutamate, Kainate, Status epilepticus.



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Several rodent models based on chemoconvulsant exposure or electrical stimulation use a lengthy period of status epilepticus to generate the development of spontaneous

recurrent seizures. This duration of status epilepticus is known to have an important effect on the experimental outcome.^{1,2} Motte 1998¹ found that heat shock protein 72

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KEY POINTS

- The Hellier Dudek model applied with 45 min status epilepticus duration generates a mild-to-moderate range of metabolic injury as measured by MRS
- There was no apparent difference in these animals based on kainate dosing or seizure count
- A multivariate cluster analysis of MRS changes performed 3 days after the status epilepticus segregated the animals into mild and moderate injury groups
- Three weeks after status epilepticus, cerebral metabolic changes consistent with the 3-day segregation of mild or moderate injury were found
- Histologic assessment 3 weeks after status epilepticus showed differences in neuronal loss and gliosis that was also consistent with the 3-day cluster analysis

(HSP72) staining appeared in the dentate gyrus only after 40–50 min of status epilepticus, with subsequent appearance in cortex coincident with recurrent motor seizures. Using an electrical dentate gyrus stimulation protocol, Norwood 2010² reported a similar 40–50 min delay with hilar Fluoro-Jade B staining. It is unclear whether such nonlinear behavior relates to human epidemiologic data (e.g., does the duration of status in febrile seizures influence the probability of development of epilepsy?).^{3,4} However, these experimentally observed delays have raised the notion that in rat models, different physiologic or pathophysiologic processes are provoked with progressively longer periods of status epilepticus.

In this report we used magnetic resonance spectroscopy (MRS) with a multivariate analysis approach to evaluate the metabolic changes in the setting of variable injury. This metabolomics approach uses the numerous measurements commonly available from MRS without bias to identify the programmatic changes that may be provoked by the seizures. Given that earlier studies have found substantial metabolic changes during the acute postseizure and epileptogenesis period,^{5,6} this approach may show coherent changes between the metabolites that reflect their known roles in neuronal and glial compartments. *N*-Acetyl aspartate (NAA) has been closely identified with neuronal mitochondrial function,⁷ whereas glutamate is the dominant excitatory neurotransmitter and intermediate in the generation of ATP within the neuronal-glial metabolic cycle.^{8,9} Although there is extensive contact between the neurotransmitter and metabolic pools of glutamate,¹⁰ the amount of extracellular neurotransmitter glutamate is small ($<3 \mu\text{M}^{11}$), and thus the large majority arises from intracellular pools of glutamate. γ -Aminobutyric acid (GABA), which provides important inhibitory balance to neurotransmitter glutamate (Glu), has a comparatively smaller direct role in energy

metabolism than glutamate¹² and is found primarily in GABAergic neurons. For the glial biomarkers, increased myo-inositol (Ins/tCr) and glutamine (Gln/tCr) have been linked with reactive astrocytic activation consistent with the known postseizure changes in osmolarity and increased glutamate clearance.^{5,13,14} In several of these studies and as discussed by Connett,¹⁵ metabolite ratios taken relative to total creatine (tCr) provide an informative assessment of metabolites that interact with ATP and are supported by the creatine kinase system. These measurements are thus considered not as absolute concentrations but rather as an index of its function that needs to be compared to control.

The Hellier Dudek incremented kainate model^{16,17} is based on 3 h of status epilepticus to consistently generate recurrent seizures and temporal lobe epilepsy pathology. In this study, we take the Hellier Dudek approach applied with a short status epilepticus period of 45 min. With this time frame, we are very close to the apparent pivot point found by Motte 1998 and Norwood 2010. Furthermore, given the known variation due to the bioavailability of intraperitoneal kainate injections (e.g., resulting from differences in local kainate absorption and brain penetration), we hypothesize that a 45-min Hellier Dudek model will generate a spectrum of injury. This report describes results from in 21 kainate-treated rats, at 3 days and 3 weeks post-status epilepticus and 10 control rats. In a subgroup of animals, we obtained histology with Nissl staining after the 3-week time point to provide histologic context for the metabolic measurements.

METHODS

Animal model

A short version of the Hellier Dudek^{16,17} incremented kainate (KA) model of temporal lobe epilepsy was used. Male Sprague-Dawley rats (180–200 g) were housed individually and handled daily during a 5-day acclimation period until model preparation. At the time of injection, rats weighed 212.8 ± 10.0 g (mean and standard deviation). Animals were injected intraperitoneally and hourly with 5 mg/kg kainate prepared in sterile saline until a stage 3/4/5 seizure (Racine 1972¹⁸) was elicited. The timing of status epilepticus (SE) started after this first stage 3/4/5 seizure and ended 45 min after this first seizure (typical duration of process for injection and status, 3–4 h). Figure 3 shows the total doses of kainate. Control rats were treated equivalently with sterile saline. Seizures were terminated with subcutaneous 20 mg/kg diazepam, which eliminated all behavioral seizures and resulted in apparent sedation. Rats were then given 5 ml warmed subcutaneous Lactated Ringer's solution and returned to their home cage. After SE, rats were weighed daily and given 5 ml warmed Lactated Ringer's and moistened rat chow to facilitate recovery. Up to the completion of imaging, we experienced a mortality rate of 14%, which is consistent with the anticipated 15% projected for this model.¹⁶ All animal experiments were approved by

the institutional animal care and use committee at the University of Pittsburgh (PHS Assurance A3187-01) and Carnegie Mellon University and were conducted in accordance with the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals. No adverse events were noted during this study.

A total of 21 kainate-treated animals were studied, of which 19 survived to complete the 3-week post-status imaging study. In addition, 10 control rats were studied. Two imaging studies were performed: 3 days after SE and 3 weeks after SE. All values are reported as mean and standard deviation. Control rats weighed 246.2 ± 11.7 g at 3 days and 382 ± 27.2 g at 3 weeks, whereas the 45-min kainate-treated animals weighed 221.8 ± 10.7 and 372.9 ± 22.3 g at 3 days and 3 weeks, respectively. All imaging studies were performed between 8 a.m. and 3 p.m.. Rats were briefly anesthetized with isoflurane, intubated, and secured in a cradle for MR imaging. Anesthesia was maintained by adjusting isoflurane 2%, N₂O at 50 PSIG and O₂ at 200 pounds per square inch gauge. A rectal thermal probe monitoring temperature was linked with feedback control to a warmed air source to maintain a 37°C body temperature. A subset of eight kainate-treated animals and four controls were evaluated after the 3-week imaging study with Nissl histologic analysis.

Magnetic resonance

A Bruker Biospec 7T 40 cm horizontal MR system was used with a 72-mm volume transmit coil and two-element receiver array. After initial three-direction localizer acquisitions, T₂-weighted RARE (rapid acquisition with relaxation enhancement multiple spin echo) acquisitions were acquired for optimal hippocampal slice positioning (Fig. 1A). Single-voxel point resolved spectroscopy (PRESS) 8 μ l ($2 \times 2 \times 2$ or $2 \times 1.8 \times 2.2$ mm³) acquisitions (17 min/voxel) at echo time (TE) 10 msec/TR 1.8 s were performed separately over the left and right dentate gyrus and CA3 regions of the hippocampus. Water suppression was performed using a VAPOR sequence. Linear combination model (LCM)¹⁹ analysis was performed for determination of the metabolite measurements, taken as a ratio to total creatine. Analyses were performed without identification as to animal grouping, and metabolites with a Cramer-Rao lower bound >15% were omitted from further analysis. At TE = 10 msec, the metabolite ratios are minimally affected by transverse relaxation values (for a typical T₂ of 130 msec, this is 8%) but are affected by T₁ and should largely be compared between control and experimental groups. For each animal, the homologous left and right measurements were averaged.

Cluster analyses

To assess the metabolic changes induced in this protocol, a multivariate analysis was performed. Initially, a hierarchical clustering was performed on the 3-day MRS data, with

the 21 kainate-treated animals. Eight MRS-identified compounds were used: NAA, Glu, Ins, Gln, GABA, Glc, and Lac, all taken as a ratio to total Cr. This exploratory multivariate approach uses the MRS data to define a linear distance between all possible pairs of animals, and then groups the animals according to their distance values into "agglomerative" clusters to minimize the variance of distances within any given cluster. These subgroups were then evaluated for their group metabolic characteristics. To further validate the clustering process, a machine learning approach was used with all of the clustered animal data to independently classify each animal using a leave-one-out analysis. As the definition of the clusters was determined by the MRS data, effectively all the spectroscopic data analysis was blinded.

Comparing the three groups (control and two kainate clusters), analysis of variance (ANOVA) statistics with Dunn-Sidak multiple comparisons testing correction was calculated, accepting significance at $p < 0.05$. All analyses were calculated using MATLAB 2013b (MathWorks, Natick, MA, U.S.A.) and Microsoft Excel. R1.1 A separate *t*-test was performed between the control and grouped kainate animals, with significance accepted at $p < 0.05$.

Histology

Eight kainate-treated and four control brains were taken after the 3-week imaging time point to provide histologic context to the spectroscopy. This was performed by transcatheter perfusion with phosphate-buffered saline pH 7.4 followed by 4% buffered paraformaldehyde. After overnight postfixation, brains were cut on a Vibratome into 50- μ m-thick serial sections and stored in a cryoprotectant solution until staining. At 300- μ m intervals through the dorsal hippocampus, sections were stained with cresyl violet, which stains the Nissl substance in all neurons and the DNA of all nuclei including glia. Stained sections were evaluated with bright-field microscopy using a subjective semiquantitative ordinal scale to indicate the degree of neuronal loss in the separate subregions of the dorsal hippocampus relative to controls. Although the analysis of the semiquantitative histologic was performed with knowledge of the total animal group proportions, specific samples were blinded.

RESULTS

Spectra and group results

Figure 1A,B show the positions of the dentate gyrus (DG), CA3 voxels, and the performance of the LCM analysis for a 3-day animal. As a group at 3 days, all 45-min rats showed significantly decreased NAA/tCr, increased Ins/tCr, Gln/tCr and Lac/tCr in comparison to control (Fig. 3). These changes are consistent with the occurrence of seizures, with evidence for neuronal mitochondrial injury (decreased NAA/tCr), glial activation with osmotic changes

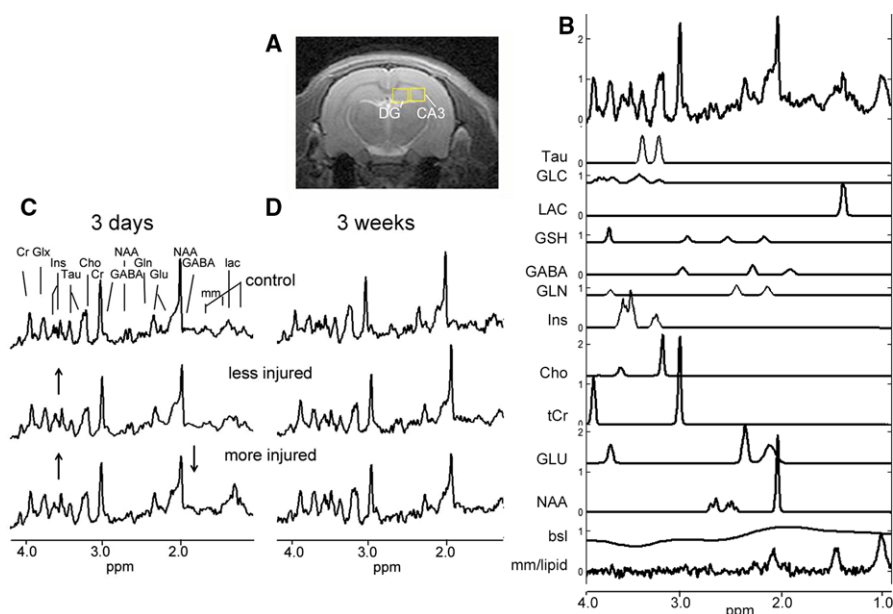


Figure 1.

Scout (**A**) showing typical loci for the dentate gyrus (DG) and CA3 (CA) voxels. (**B**) LCM analyzed DG spectrum from a KA-treated animal, showing individual metabolites. Corresponding spectra acquired at 3 days (**C**) and 3 weeks (**D**) are shown from single (all survival) rats, showing matched control and two KA rats. Identified metabolites are shown in the control 3-day spectrum. The 3-day spectra from the two KA rats show variability, consistent with a “less” and “more” injured grouping. The arrows on the “more injured” 3-day spectra show a decreased NAA and increased Ins. The “less injured” 3-day spectra show primarily the increased Ins.

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and glutamine synthetase activation (Ins/tCr, Gln/tCr respectively), and glycolytic energy production (Lac/tCr) commonly seen in seizures and post-status. However, as anticipated, there was variability with the kainate-treated animals, seen from the 3-day and 3-week spectra (see Fig. 1C,D). Because data from the CA3 locus did not substantially change these results, these data are available in Supporting Information.

Cluster analysis

With the possibility that a wide range of injuries might be identified with the 45-min status period, we evaluated the animals using a hierarchical cluster approach on the 3-day MRS results. The dentate gyrus voxel was used, as this receives much of the hippocampal input and is a known locus for establishment of recurrent excitatory circuits.²⁰ The analysis was performed using a two-cluster structure where each animal is one data point (averaging the left and right). Figure 2A shows the 3-day two-cluster result, separating the 21 kainate-treated animals into groups of 6 and 15. For clarity, the three-dimensional plot is shown with two perspectives, showing the metabolite differences in NAA/tCr, Glu/tCr, and GABA/tCr. Control data (blue) are also included on this plot for comparative purposes. The largest change was identified in the 6 (red) animals that had NAA/tCr values below the value of 1.0, whereas the 15 (green) animals show a small increase in Glu/tCr. The 6 animals

were identified as kainate group KM (“more” injured), and the 15 as kainate group KL (“less” injured). The two-cluster analysis taken with the CA sector voxel could segregate five of the six same animals (data not shown).

Given that the cluster analysis on the 3-day MRS data was able to segregate the animals, it was of interest to understand whether the same clusters could be used in a machine-learning approach to discriminate between the animals. With a leave-one-out method for cross validation, each animal was tested as a new sample point against the remainder of the animals. The cross validation error with this approach was small, at 3.23%, consistent with the data shown in Figure 2A with the relatively large distances between the three clusters, the largest distance parameter being NAA/tCr.

Metabolic differences between the clusters

The cluster analysis of the kainate-treated animals at 3 days identified 6 and 15 animals in terms of their metabolic patterns, suggesting two levels of injury. The $n = 6$ KM group showed a 28.7% decline in NAA/tCr compared to the 10 controls animals, whereas the $n = 15$ KL group showed a 6.0% decline. From this segregation, the KM group generally shows worse changes in the other metabolic parameters. This is manifest with a greater decline in Glu/tCr, and larger changes in glial biomarkers, with increased inositol Ins/tCr, glutamine Gln/tCr, and also increased lactate Lac/tCr (Fig. 3).

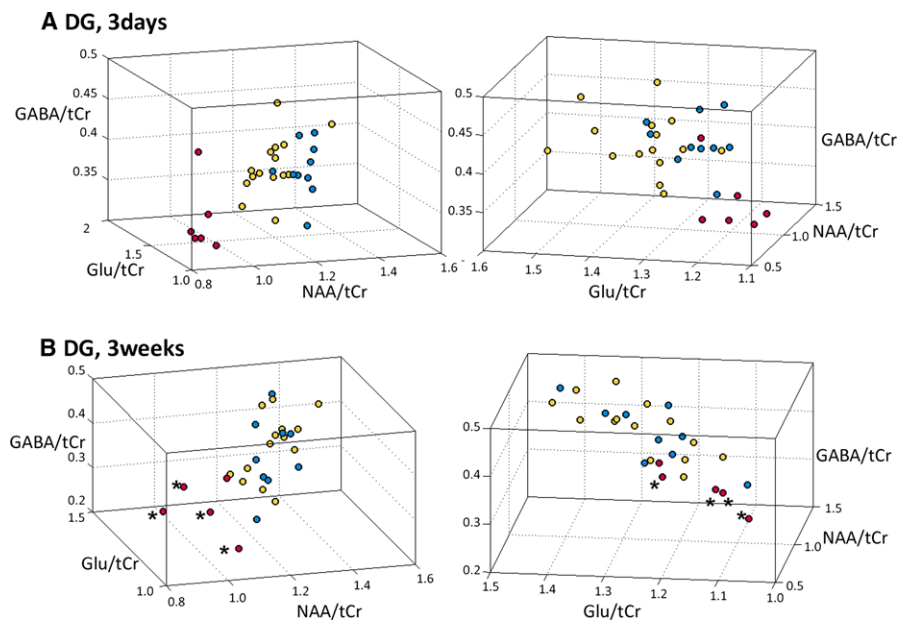


Figure 2.

Cluster analysis of kainate-treated animals ($n = 21$ total), showing three-dimensional plots of NAA/tCr, Glu/tCr, and GABA/tCr from the DG locus. For comparison, control animals are also shown. **(A)** Three-day data; **(B)** 3-week data. For both **(A and B)**, the plots are shown from two rotated perspectives to show the relationships between NAA/tCr with GABA/tCr (left) and between Glu/tCr with GABA/tCr (right) more clearly. The largest separation between KL and KM is in the 3-day data with NAA/tCr (panel **A**, left). Red represents the KM group; green are the KL group; blue are the control group. Four (asterisked) of the 5 KM animals in the 3-week data are animals that were segregated by a separate cluster analysis of 3-week data alone.

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The KL group showed similar but milder changes, with the exception of the 3-day glutamatergic biomarker Glu/tCr, which increased rather than decreased. For the 3-week studies, one animal from each of the groups (control, KM, KL) did not successfully complete the study. Both the KM and KL groups exhibited recovery after the status period such that the differences in metabolites were generally smaller at 3 weeks than at 3 days. For NAA/tCr, the KL group had returned to control values at 1.28 ± 0.12 , whereas in the KM group it stayed depressed at 1.00 ± 0.12 (control, 1.30 ± 0.15). The 3-week KM group also continued to show a significantly increased Ins/tCr and a decreased Glu/tCr. In contrast, the 3-week KL group had largely normalized all of its metabolic parameters, including NAA/tCr, Ins/tCr, and Gln/tCr, with Glu/tCr decreasing to a normal level. To show the multivariate evolution of these data, Figure 2B shows the same three-dimensional plot of NAA/tCr, Glu/tCr, and GABA/tCr tracking the KL, KM, and control animals into the 3-week study. Consistent with Figure 3, the KM group displays persistently low NAA/tCr, with simultaneous declines in Glu/tCr and GABA/tCr. A separate cluster analysis of the 3-week dentate gyrus data co-identified four of the five KM animals as separate from the other animals; these animals are identified by asterisks in Figure 2B.

Histology

To provide histologic context to the KM and KL groups, a subset ($n = 8$) of animals from the KM ($n = 4$) and KL ($n = 4$) groups was analyzed with Nissl staining in comparison to $n = 4$ controls. Table 1 summarizes these data with their 3-day dentate gyrus spectroscopic data. Animals from the KL group have a dorsal hippocampus that is largely intact in all subregions, although two animals in this group showed minimal neuron loss in the hilus. In contrast, the KM animals show significant neuronal loss most apparent in area CA1 and in some cases extending into the subiculum. In these regions, neurons of the pyramidal cell layers are largely replaced by glia. Increased glial proliferation is also seen in the adjacent stratum radiatum. Neuronal loss is evident in the hilus and CA3 regions. In general, the patterns on neuronal loss on the left and right sides are similar. This is evident in the photo micrographs shown in Figure 4 from KL 77 and 92, KM 81 and 87 which are from the animals that showed the least and most damage from each of the two groups respectively. In the KM group, the two animals with the greatest dorsal gliosis (80 and 81) had the lowest NAA/tCr values. In the KL group, the two animals with any degree of abnormality (88 and 92) exhibited the greatest Gln/tCr but also the largest Glu/tCr values.

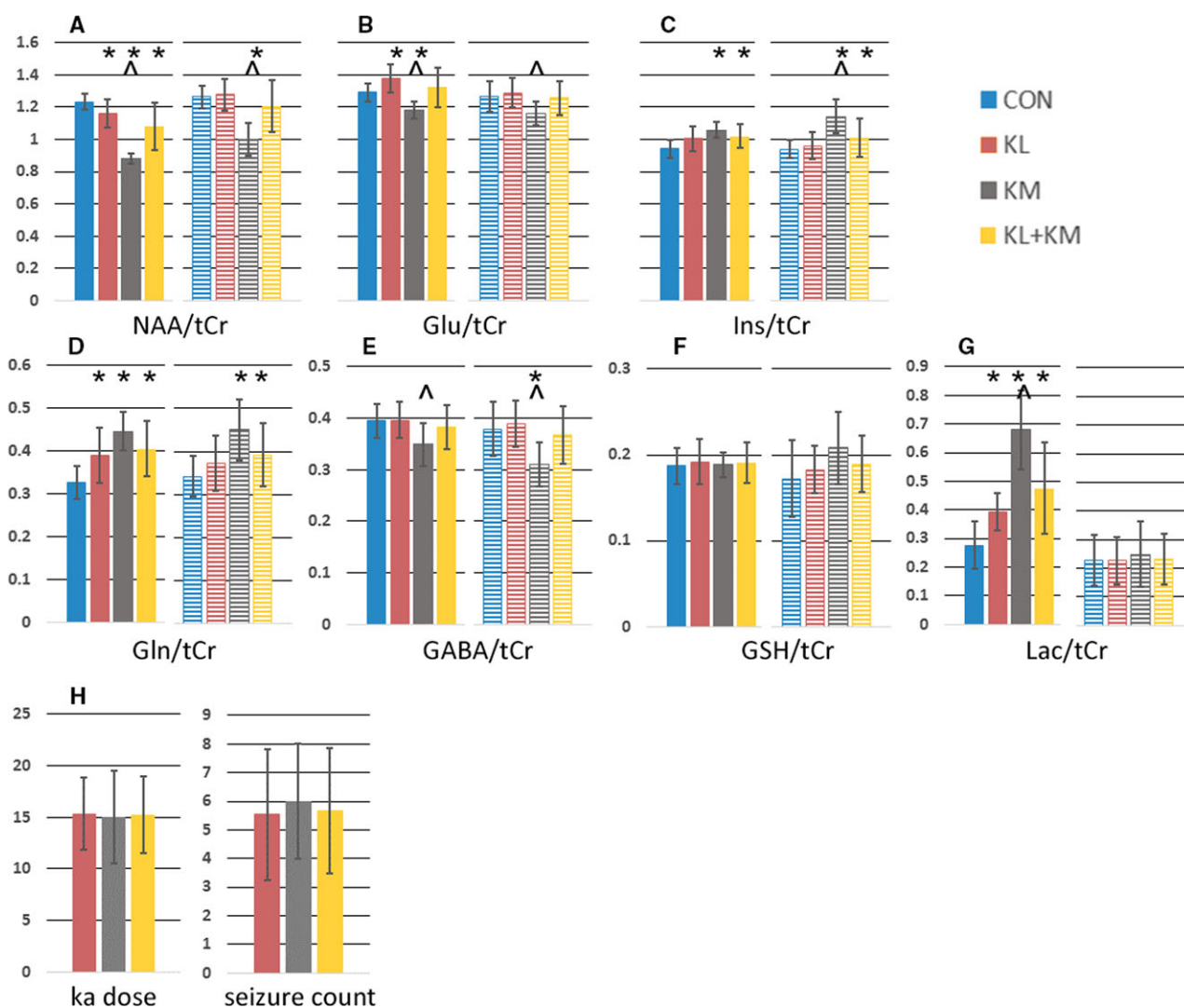


Figure 3.

Bar plots showing the mean values and standard deviations from the two cluster (KM $n = 6$, KL $n = 15$) and control ($n = 10$) groups from 3 days (solid bars) and the same animals from 3 weeks (KM, $n = 5$; KL $n = 14$; control $n = 9$; cross-hatched bars). The KM and KL groups are also combined to show the group statistics from the summed KA-treated group. All data are from the DG locus. (A) NAA/tCr; (B) Glu/tCr; (C) Ins/tCr; (D) Gln/tCr; (E) GABA/tCr; (F) GSH/tCr; (G) Lac/tCr; (H) KA dosing and seizure counts from model preparation. For the KM, KL, and control groups, significance testing is performed using a one-way ANOVA with multiple testing corrections (Dunn-Sidak), $p < 0.05$. *Significant differences $p < 0.05$ between control and KM or KL group; ^Significant difference $p < 0.05$ between KM and KL. For the combined KM + KL versus control groups, significance testing is performed using a t -test ($p < 0.05$); *Significant differences $p < 0.05$ between the control and KM + KL groups.

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DISCUSSION

Cluster analysis of a variable model

These data show that the short Hellier Dudek model generates a wide range of metabolic dysfunction, ranging from mild to more severe injury. It is also consistent with the practice of using longer periods of status to generate a high rate of recurrent seizures. A multivariate cluster analysis is able to separate within the kainate-treated group to define more injured versus less injured (KM vs. KL respectively) animals, even though there was no apparent

difference in seizure severity or required kainate doses in these groups. The relevance of this analysis is supported by the Nissl analysis from the dorsal hippocampus and by the consistency and persistence of these changes at 3 weeks after status.

Metabolic shifts in the cluster groups

It is worth considering the magnitude of differences relative to control in the two groups. At 3 days, the KL group exhibits a 6.0% decline in NAA/tCr accompanied by a 6.7% increase in Glu/tCr, retrospectively hypothesized as a

Table 1. Semiquantitative Nissl-staining analysis

Rat no.	GC	Hilus	CA3 prox	CA3 distal	CA1	Sub	Gliosis	NAA	Glu	Ins	Gln	GABA
								1.23 ± 0.08	1.29 ± 0.08	0.94 ± 0.07	0.33 ± 0.07	0.40 ± 0.05
KL76	0	0	0	0	0	0	0	1.08	1.22	1.17	0.33	0.40
KL77	0	0	0	0	0	0	0	1.19	1.33	0.88	0.26	0.43
KL88	0	+	0	0	0	0	0	1.11	1.43	0.92	0.41	0.38
KL92	0	+	0	0	0	0	0	1.26	1.57	0.97	0.48	0.37
KM80	0	++	+	0	++	+	+++	0.83	1.11	1.02	0.49	0.34
KM81	0	++	+	+++	++	0	+++	0.86	1.18	1.11	0.43	0.33
KM87	0	0	+	++	++	0	++	0.92	1.14	1.04	0.47	0.32
KM98	0	+++	++	0	0	0	+	0.90	1.17	1.01	0.37	0.36

GC, granule cell layer; CA3 px, CA3 proximal; CA3 dist, CA3 distal; Sub, subiculum.
 All animals were killed after the 3-week imaging study, and preserved using 4% buffered paraformaldehyde. Data are presented as the differences compared to control. Left seven columns: histologic region of analysis.
 Right five columns: corresponding metabolite ratios taken to total creatine from the dentate gyrus; left/right values averaged per animal. Control MRS values are shown in top header row. Top four rows KL; bottom four rows KM.

metabolically “healthy” response to SE. This is accompanied by a 6.7% rise in Ins/tCr and 19.1% rise in Gln/tCr. 3 weeks later; the metabolic changes in this group return to normal. It would appear that although the 3-day changes are significant, at 3 weeks, the animals display metabolic recovery with minimal histologic changes.

In the KM group, there are larger changes in both NAA/tCr (−28.7%) and Glu/tCr (−8.6%), whereas Ins/tCr (12.6%) and Gln/tCr (36.5%) increase, consistent with a broad injury across both neuronal and glial compartments. There is a more than doubling of the tissue Lac/tCr (+147% rise) in these animals. Considering that these studies were acquired 3 days after status, these are persistent metabolic changes, and may be a result of permanent injury, prolonged recovery, or responses that reflect post-status processes. At 3 weeks, the KM group does not exhibit normalization of Glu/tCr, NAA/tCr; Ins/tCr and Gln/tCr remain increased. The histologic data in the KM group bear out substantial injury with gliosis and neuronal loss.

The glutamate/tCr (Glu/tCr) parameter is of particular interest given its splay between the KL and KM groups. The 3-day KL group displayed increased Glu/tCr (1.38 ± 0.12), whereas the 3-day KM group showed decreased Glu/tCr (1.18 ± 0.10; control 1.29 ± 0.08, both significantly different from control). It is possible that T2 relaxation changes could contribute to this observation; however, use of a short 10-msec TE sharply limits such effects. Thus the alternative needs to be considered, that is, that the observed Glu/tCr increase in KL is a response to excitatory injury, supporting increased resistance to ictogenic insult.²¹ There is evidence for this, such as excitatory synapse redistribution,²² increased gene expression for inflammation, oxidative stress, and calcium signaling.²³ Furthermore, given that glutamate is an intermediate in energy generation, increased pool size for energy production could result in increased glutamate content and thus be a potentially “healthy” response to a (presumptively mild) seizure insult. Three weeks later both Glu/tCr and NAA/tCr normalize, this being consistent with the overall healthier KL group, and its fewer histologic abnormalities. In contrast, the KM group’s decrease in NAA/tCr is three-fold higher than the drop in Glu/tCr and may reflect permanent injury, consistent with its histology and the persistently low NAA/tCr (−20.9% compared with control) at 3 weeks.

From a glial perspective, the KL data define a range of variability for Ins/tCr and Gln/tCr from which metabolic recovery appears possible. As is well known, glutamine is in balance between several systems of glutamate neurotransmission, ammonia homeostasis, and oxidative flux. Thus there are many factors that may influence Gln/tCr. From the KM group, our observations of sizable increases in Gln/tCr (36.5%, approximately twofold larger than the KL group at 19.1%) is consistent

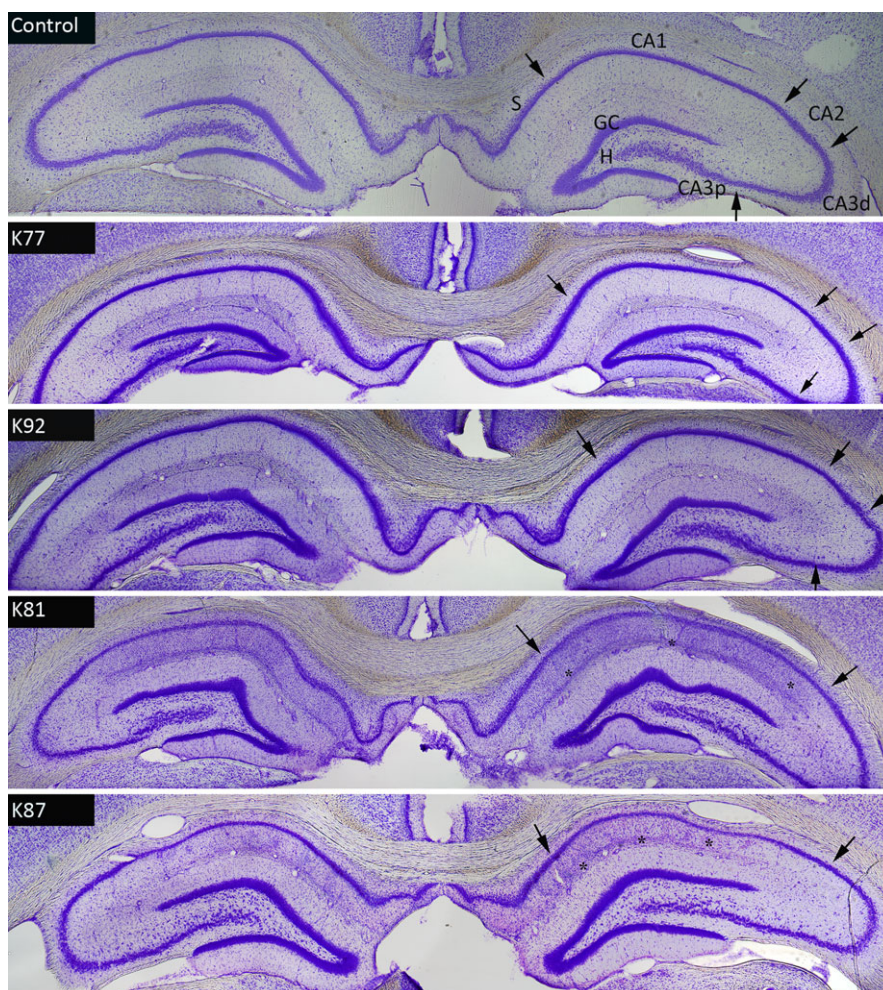


Figure 4.

Nissl-stained sections through the dorsal hippocampus for a control and two KL and KM animals (see Table I for matching identifiers). The arrows separate the various CA sectors. The neuronal loss in CA1 is marked for the bottom two KM animals, and asterisks mark the extensive gliosis in the stratum radiatum. S, subiculum; GC, granule cell layer; H, hilus, CA3p, CA3 proximal, CA3d, CA3 distal.

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with results of Van der Hel²⁴ and may be due partially to conversion of excitatory glutamate (which decreased by 8.6%) into glutamine, given that the normal Glu-Gln neurotransmission cycling is most likely not occurring in the injured KM group.²⁵ This dysfunctional cycling is likely to also contribute to observations from human temporal lobe epilepsy cases showing normal levels of extracellular glutamine even in the face of decreased glutamine synthetase and known gliosis.^{26,27}

Caveats

The major caveat in this work is that of the use of metabolite ratios with total creatine. The theoretical basis for its use has been discussed by Connett,¹⁵ who proposed it as a thermodynamically useful normalization in the analysis of bioenergetic processes in tissues that use the creatine kinase system as a mechanism for ATP buffering. In this analysis, a ratio with total creatine provides a measure

of its dispersion²⁴ from baseline, which we would argue that for the metabolites discussed in this work is highly pertinent. Nonetheless, if the creatine transport system itself becomes distorted in the post-status period, such ratios will be problematic. There are relatively few data on this issue, but Gupta et al.²⁸ found no significant change in total creatine at 3 days in an acetylcholinesterase model of seizures; and Melo,²⁹ using a pilocarpine model, similarly found no significant changes at 1 month in chronically epileptic rats. We would argue that the 45-min duration of status is comparatively brief and believe that as biomarkers of physiologic functions, the observed metabolic changes stand.

CONCLUSIONS

Overall, these results are consistent with programmatic metabolic processes that are variably instigated by the 45-

min status, consistent with a range between extreme versus pathologic neurotransmission. The generation of the two discrete groups (rather than a conglomeration of one injured group) is the notable observation. That these metabolically identified groups appear to propagate into the 3-week time point argues for the notion that the short <1 h status period seems to generate a tipping point, as discussed earlier. It will be of interest to understand whether these metabolic changes, reflecting their neuronal and glial sources, link with cellular processes of gene expression, remodeling, and inflammation, which may be related to the ultimate development of spontaneous recurrent seizures.

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DISCLOSURE

None of the authors has any conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Group means of metabolite ratios from CA3 voxels.